

PATENT ABSTRACTS OF JAPAN

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C12N 9/10

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(22)Date of filing : 20.02.2001

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(54) PLANT CELL HAVING ANIMAL-TYPE SUGAR CHAIN-ADDING FUNCTION

(57)Abstract:

PROBLEM TO BE SOLVED : To provide a plant cell having an animal-type sugar chain.

SOLUTION : A plant cell having an animal-type sugar chain-adding function. The plant cell is prepared by introducing a gene encoding an animal-derived enzyme which can transfer N-acetylglucosamine into a mannose residue of a sugar chain contained in a sugar protein.

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(54) 【発明の名称】 動物型糖鎖付加機能を持つ植物細胞

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N., Pierce, M. Isolation, Characterization, and Expression of a cDNA Encoding N-Acetylglucosaminyltransferase V. (1993) J. Biol. Chem. 268, 15381-1538

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o F u i a N F R Q O

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表2. 使用菌株

株	遺伝子型および特徴	参考文献
Escherichia coli JM109	recA1, endA1, gyrA96, thi, hsdR 17, supE 44, relA1, Δ (lac-proAB) /F' [traD 36, proAB', lacI ^q , lacZΔM15]	Yanisch-Perron C ら (*)
Agrobacterium tumefaciens LBA4404	Str ^r , Rif ^r バイナリーベクターのT-DNA領域の 移入を容易にするために必要な、 トランスに作用する病原性 (virul ence) 機能を有する	Jen, G.C. ら (**)

(*) Yanisch-Perron C, Vieira J, Messing J. Improved M13 phage cloning
g vectors and host strains: nucleotide sequences of the M13mp18 and pUC1
9 vectors. (1985) Gene 33, 103-119

(**) Jen, G.C., Chilton, M. D. The right border region of pTiT37
T-DNA is intrinsically more active than the left border region in
promoting T-DNA transformation. (1986) Proc. Natl. Acad. Sci. USA. 83,
3895-3899

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表3. 使用プラスミド

プラスミド	遺伝子型および特徴	参考文献
pBI121		Jfferson, R. A. ら (*)
pRK2013	Km ^r 3 親交配により、E. coli から A. tumefaciens にバイナリーベクター中の構造物を移動するために用いた接合プラスミド	Bevan, M. (**)

(*) Jfferson, R. A., Kavanagh, T. A., Bevan, M. W. GUS fusion : β -glucuronidase as a sensitive and versatile gene fusion in higher plants. (1987) EMBO. J. 6, 3901-3907

(**) Bevan, M. Binary Agrobacterium vectors for plant transformation. (1984) Nucleic Acids. Res. 12, 8711-8721.

hia coli JM109 A w PRK2013] ~ B
 Escherichia coli A » E 20mg/l J i y O O X T z P U D ^ o R
 C V 2 YT | n - R V A P *
 E LEA4044 X g 100 mg/l A @ i P U D P D Neasy Plant Mini Kit (Q
 s 100 mg/l 2 YT | 28 A Q { IAGEN) p c ~ s ^ o R
 %B c ~ t % a A » c ~
 y O O X O z e | { t ~
 L A W (10,000 rpm, 1 min) A » ~ y O O X U z i P U D
 YT | n - P æ 2 YT | n
 % B V c ^ [u ~ w 10
 E t A I
 A » S ° R ¶ n z
 « 28 - | { • Ø - ~
 ' A O o N e E
 a f V | n a - P ° B %
 L A R ¶ 2 YT | n a h z
 Q æ | { % a A P
 y O O X P z P S D ^
 d An, G., Ebert, P. R., Mitra,
 A. and Ha, S. B. (1988) Binary vectors. In plant M 20
 olecular Biology manual, A3, 1-19, AcademicDordrec
 ht j] ~
 y O O X Q z i P S D P D
 R O O } C [t 5 ml
 E A ^ 25 27 • x 20rpm a
 x - | { s ^ % B V æ
 , ' A
 y O O X R z i P S D Q D
 R ¶ n z
 o N e a h n v X 30
 o N e EBA4044)100 I ~ A | { S
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 - ^ - a æ a A V | t
 S 1,000 rpm, 5 min) L a
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 f • Ø - ~ L E
 s ^ % B A O o N e
 R | { 1 | n 50
 kg/l l A N 100 kg/l l > "
 V 40
 T A ^ 2 a J
 A R ¶ n z fl 15 f V | n
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 DNeasy Plant Mini Kit (QIAGEN) p c B ^
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(14)														J	Q	O	O	Q	Q	Q							
25							26																				
90							95							100													
aag	gtg	gac	aat	ctt	gtt	gtc	aat	ggc	acc	gga	aca	aac	tca	acc	aac	509											
Lys	Val	Asp	Asn	Leu	Val	Val	Asn	Gly	Thr	Gly	Thr	Asn	Ser	Thr	Asn												
105							110							115													
tcc	act	aca	gct	gtt	ccc	agc	ttg	gtt	gca	ctt	gag	aaa	att	aat	gtg	557											
Ser	Thr	Thr	Ala	Val	Pro	Ser	Leu	Val	Ala	Leu	Glu	Lys	Ile	Asn	Val												
120							125							130													
gca	gat	atc	att	aac	gga	gct	caa	gaa	aaa	tgt	gta	ttg	cct	cct	atg	605											
Ala	Asp	Ile	Ile	Asn	Gly	Ala	Gln	Glu	Lys	Cys	Val	Leu	Pro	Pro	Met												
135							140							145							150						
gac	ggc	tac	cct	cac	tgt	gag	gga	aag	atc	aag	tggt	atg	aaa	gac	atg	653											
Asp	Gly	Tyr	Pro	His	Cys	Glu	Gly	Lys	Ile	Lys	Trp	Met	Lys	Asp	Met												
155							160							165													
tggt	cgt	tca	gat	ccc	tgc	tac	gca	gac	tat	gga	gtg	gat	gga	tcc	acc	701											
Trp	Arg	Ser	Asp	Pro	Cys	Tyr	Ala	Asp	Tyr	Gly	Val	Asp	Gly	Ser	Thr												
170							175							180													
tgc	tct	ttt	ttt	att	tac	ctc	agt	gag	gtt	gaa	aat	tggt	tgt	cct	cat	749											
Cys	Ser	Phe	Phe	Ile	Tyr	Leu	Ser	Glu	Val	Glu	Asn	Trp	Cys	Pro	His												
185							190							195													
tta	cct	tggt	aga	gca	aaa	aat	ccc	tac	gaa	gaa	gct	gat	cat	aat	tca	797											
Leu	Pro	Trp	Arg	Ala	Lys	Asn	Pro	Tyr	Glu	Glu	Ala	Asp	His	Asn	Ser												
200							205							210													
ttg	gcgt	gaa	att	cgt	aca	gat	ttt	aat	att	ctc	tac	agt	atg	atg	aaa	845											
Leu	Ala	Glu	Ile	Arg	Thr	Asp	Phe	Asn	Ile	Leu	Tyr	Ser	Met	Met	Lys												
215							220							225							230						
aag	cat	gaa	gaa	ttc	cgt	tggt	atg	aga	cta	cgt	atc	cgt	cga	atg	gct	893											
Lys	His	Glu	Glu	Phe	Arg	Trp	Met	Arg	Leu	Arg	Ile	Arg	Arg	Met	Ala												
235							240							245													
gac	gca	tggt	atc	caa	gca	atc	aag	tcc	ctg	gca	gaa	aag	cag	aac	ctt	941											
Asp	Ala	Trp	Ile	Gln	Ala	Ile	Lys	Ser	Leu	Ala	Glu	Lys	Gln	Asn	Leu												
250							255							260													
gaa	aag	aga	aag	cgt	aag	aaa	gtc	ctc	gtt	cac	ctg	gga	ctc	ctg	acc	989											
Glu	Lys	Arg	Lys	Arg	Lys	Lys	Val	Leu	Val	His	Leu	Gly	Leu	Leu	Thr												
265							270							275													
aag	gaa	tct	gga	ttt	aag	att	gca	gag	aca	gct	ttc	agt	ggt	ggc	cct	1037											
Lys	Glu	Ser	Gly	Phe	Lys	Ile	Ala	Glu	Thr	Ala	Phe	Ser	Gly														

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Phe	Lys	Lys	Thr	Leu	Gly	Pro	Ser	Trp	Val	His	Tyr	Gln	Cys	Met	Leu	
360						365					370					
cga	gtc	ctt	gat	tca	ttt	ggt	act	gaa	ccc	gaa	ttt	aat	cat	gca	aat	1325
Arg	Val	Leu	Asp	Ser	Phe	Gly	Thr	Glu	Pro	Glu	Phe	Asn	His	Ala	Asn	
375					380					385					390	
tat	gcc	caa	tcg	aaa	ggc	cac	aag	acc	cct	tgg	gga	aaa	tgg	aat	ctg	1373
Tyr	Ala	Gln	Ser	Lys	Gly	His	Lys	Thr	Pro	Trp	Gly	Lys	Trp	Asn	Leu	
				395					400					405		
aac	cct	cag	cag	ttt	tat	acc	atg	ttc	cct	cat	acc	cca	gac	aac	agc	1421
Asn	Pro	Gln	Gln	Phe	Tyr	Thr	Met	Phe	Pro	His	Thr	Pro	Asp	Asn	Ser	
		410						415					420			
ttt	ctg	ggg	ttt	gtg	gtt	gag	cag	cac	ctg	aac	tcc	agt	gat	atc	cac	1469
Phe	Leu	Gly	Phe	Val	Val	Glu	Gln	His	Leu	Asn	Ser	Ser	Asp	Ile	His	
		425				430					435					
cac	att	aat	gaa	atc	aaa	agg	cag	aac	cag	tcc	ctt	gtg	tat	ggc	aaa	1517
His	Ile	Asn	Glu	Ile	Lys	Arg	Gln	Asn	Gln	Ser	Leu	Val	Tyr	Gly	Lys	
	440				445					450						
gtg	gat	agc	ttc	tgg	aag	aat	aag	aag	atc	tac	ttg	gac	att	att	cac	1565
Val	Asp	Ser	Phe	Trp	Lys	Asn	Lys	Lys	Ile	Tyr	Leu	Asp	Ile	Ile	His	
455				460						465				470		
aca	tac	atg	gaa	gtg	cat	gca	act	gtt	tat	ggc	tcc	agc	aca	aag	aat	1613
Thr	Tyr	Met	Glu	Val	His	Ala	Thr	Val	Tyr	Gly	Ser	Ser	Thr	Lys	Asn	
			475					480					485			
att	ccc	agt	tac	gtg	aaa	aac	cat	ggt	atc	ctc	agt	gga	cgg	gac	ctg	1661
Ile	Pro	Ser	Tyr	Val	Lys	Asn	His	Gly	Ile	Leu	Ser	Gly	Arg	Asp	Leu	
		490						495				500				
cag	ttc	ctt	ctt	cga	gaa	acc	aag	ttg	ttt	gtt	gga	ctt	ggg	ttc	cct	1709
Gln	Phe	Leu	Leu	Arg	Glu	Thr	Lys	Leu	Phe	Val	Gly	Leu	Gly	Phe	Pro	
		505				510					515					
tac	gag	ggc	cca	gct	ccc	ctg	gaa	gct	atc	gca	aat	gga	tgt	gct	ttt	1757
Tyr	Glu	Gly	Pro	Ala	Pro	Leu	Glu	Ala	Ile	Ala	Asn	Gly	Cys	Ala	Phe	
	520				525					530						
ctg	aat	ccc	aag	ttc	aac	cca	ccc	aaa	agc	agc	aaa	aac	aca	gac	ttt	1805
Leu	Asn	Pro	Lys	Phe	Asn	Pro	Pro	Lys	Ser	Ser	Lys	Asn	Thr	Asp	Phe	
535				540						545				550		
ttc	att	ggc	aag	cca	act	ctg	aga	gag	ctg	aca	tcc	cag	cat	cct	tac	1853
Phe	Ile	Gly	Lys	Pro	Thr	Leu	Arg	Glu	Leu	Thr	Ser	Gln	His	Pro	Tyr	
			555					560				565				
gct	gaa	gtt	ttc	atc	ggg	cgg	cca	cat	gtg	tgg	act	gtt	gac	ctc	aac	1901
Ala	Glu	Val	Phe	Ile	Gly	Arg	Pro	His	Val	Trp	Thr	Val	Asp	Leu	Asn	
		570				575						580				
aat	cag	gag	gaa	gta	gag	gat	gca	gtg	aaa	gca	att	tta	aat	cag	aag	1949
Asn	Gln	Glu	Glu	Val	Glu	Asp	Ala	Val	Lys	Ala	Ile	Leu	Asn	Gln	Lys	
		585				590						595				
att	gag	cca	tac	atg	cca	tat	gaa	ttt	acg	tgc	gag	ggg	atg	cta	cag	1997
Ile	Glu	Pro	Tyr	Met	Pro	Tyr	Glu	Phe	Thr	Cys	Glu	Gly	Met	Leu	Gln	
	600				605					610						
aga	atc	aat	gct	ttc	att	gaa	aaa	cag	gac	ttc	tgc	cat	ggg	caa	gtg	2045

Arg Ile Asn Ala Phe Ile Glu Lys Gln Asp Phe Cys His Gly Gln Val
 615 620 625 630
 atg tgg cca ccc ctc agc gcc cta cag gtc aag ctt gct gag ccc ggg 2093
 Met Trp Pro Pro Leu Ser Ala Leu Gln Val Lys Leu Ala Glu Pro Gly
 635 640 645
 cag tcc tgc aag cag gtg tgc cag gag agc cag ctc atc tgc gag cct 2141
 Gln Ser Cys Lys Gln Val Cys Gln Glu Ser Gln Leu Ile Cys Glu Pro
 650 655 660
 tct ttc ttc cag cac ctc aac aag gac aag gac atg ctg aag tac aag 2189
 Ser Phe Phe Gln His Leu Asn Lys Asp Lys Asp Met Leu Lys Tyr Lys
 665 670 675
 gtg acc tgc caa agc tca gag ctg gcc aag gac atc ctg gtg ccc tcc 2237
 Val Thr Cys Gln Ser Ser Glu Leu Ala Lys Asp Ile Leu Val Pro Ser
 680 685 690
 ttt gac cct aag aat aag cac tgt gtg ttt caa ggt gac ctc ctg ctc 2285
 Phe Asp Pro Lys Asn Lys His Cys Val Phe Gln Gly Asp Leu Leu Leu
 695 700 705 710
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 Phe Leu Val Thr Phe Gly Phe Ile Trp Gly Met Met Leu Leu His Phe
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 Thr Ile Gln Gln Arg Thr Gln Pro Glu Ser Ser Ser Met Leu Arg Glu
 35 40 45
 Gln Ile Leu Asp Leu Ser Lys Arg Tyr Ile Lys Ala Leu Ala Glu Glu
 50 55 60
 Asn Arg Asn Val Val Asp Gly Pro Tyr Ala Gly Val Met Thr Ala Tyr
 65 70 75 80
 Asp Leu Lys Lys Thr Leu Ala Val Leu Leu Asp Asn Ile Leu Gln Arg
 85 90 95
 Ile Gly Lys Leu Glu Ser Lys Val Asp Asn Leu Val Val Asn Gly Thr
 100 105 110
 Gly Thr Asn Ser Thr Asn Ser Thr Thr Ala Val Pro Ser Leu Val Ala
 115 120 125
 Leu Glu Lys Ile Asn Val Ala Asp Ile Ile Asn Gly Ala Gln Glu Lys
 130 135 140
 Cys Val Leu Pro Pro Met Asp Gly Tyr Pro His Cys Glu Gly Lys Ile
 145 150 155 160
 Lys Trp Met Lys Asp Met Trp Arg Ser Asp Pro Cys Tyr Ala Asp Tyr

31
 165 170 175
 Gly Val Asp Gly Ser Thr Cys Ser Phe Phe Ile Tyr Leu Ser Glu Val
 180 185 190
 Glu Asn Trp Cys Pro His Leu Pro Trp Arg Ala Lys Asn Pro Tyr Glu
 195 200 205
 Glu Ala Asp His Asn Ser Leu Ala Glu Ile Arg Thr Asp Phe Asn Ile
 210 215 220
 Leu Tyr Ser Met Met Lys Lys His Glu Glu Phe Arg Trp Met Arg Leu
 225 230 235 240

 Arg Ile Arg Arg Met Ala Asp Ala Trp Ile Gln Ala Ile Lys Ser Leu
 245 250 255
 Ala Glu Lys Gln Asn Leu Glu Lys Arg Lys Arg Lys Lys Val Leu Val
 260 265 270
 His Leu Gly Leu Leu Thr Lys Glu Ser Gly Phe Lys Ile Ala Glu Thr
 275 280 285
 Ala Phe Ser Gly Gly Pro Leu Gly Glu Leu Val Gln Trp Ser Asp Leu
 290 295 300
 Ile Thr Ser Leu Tyr Leu Leu Gly His Asp Ile Arg Ile Ser Ala Ser
 305 310 315 320
 Leu Ala Glu Leu Lys Glu Ile Met Lys Lys Val Val Gly Asn Arg Ser
 325 330 335
 Gly Cys Pro Thr Val Gly Asp Arg Ile Val Glu Leu Ile Tyr Ile Asp
 340 345 350
 Ile Val Gly Leu Ala Gln Phe Lys Lys Thr Leu Gly Pro Ser Trp Val
 355 360 365
 His Tyr Gln Cys Met Leu Arg Val Leu Asp Ser Phe Gly Thr Glu Pro
 370 375 380
 Glu Phe Asn His Ala Asn Tyr Ala Gln Ser Lys Gly His Lys Thr Pro
 385 390 395 400

 Trp Gly Lys Trp Asn Leu Asn Pro Gln Gln Phe Tyr Thr Met Phe Pro
 405 410 415
 His Thr Pro Asp Asn Ser Phe Leu Gly Phe Val Val Glu Gln His Leu
 420 425 430
 Asn Ser Ser Asp Ile His His Ile Asn Glu Ile Lys Arg Gln Asn Gln
 435 440 445
 Ser Leu Val Tyr Gly Lys Val Asp Ser Phe Trp Lys Asn Lys Lys Ile
 450 455 460
 Tyr Leu Asp Ile Ile His Thr Tyr Met Glu Val His Ala Thr Val Tyr
 465 470 475 480
 Gly Ser Ser Thr Lys Asn Ile Pro Ser Tyr Val Lys Asn His Gly Ile
 485 490 495
 Leu Ser Gly Arg Asp Leu Gln Phe Leu Leu Arg Glu Thr Lys Leu Phe
 500 505 510
 Val Gly Leu Gly Phe Pro Tyr Glu Gly Pro Ala Pro Leu Glu Ala Ile
 515 520 525
 Ala Asn Gly Cys Ala Phe Leu Asn Pro Lys Phe Asn Pro Pro Lys Ser
 530 535 540
 Ser Lys Asn Thr Asp Phe Phe Ile Gly Lys Pro Thr Leu Arg Glu Leu

545 550 555 560
 Thr Ser Gln His Pro Tyr Ala Glu Val Phe Ile Gly Arg Pro His Val
 565 570 575
 Trp Thr Val Asp Leu Asn Asn Gln Glu Glu Val Glu Asp Ala Val Lys
 580 585 590
 Ala Ile Leu Asn Gln Lys Ile Glu Pro Tyr Met Pro Tyr Glu Phe Thr
 595 600 605
 Cys Glu Gly Met Leu Gln Arg Ile Asn Ala Phe Ile Glu Lys Gln Asp
 610 615 620
 Phe Cys His Gly Gln Val Met Trp Pro Pro Leu Ser Ala Leu Gln Val
 625 630 635 640
 Lys Leu Ala Glu Pro Gly Gln Ser Cys Lys Gln Val Cys Gln Glu Ser
 645 650 655
 Gln Leu Ile Cys Glu Pro Ser Phe Phe Gln His Leu Asn Lys Asp Lys
 660 665 670
 Asp Met Leu Lys Tyr Lys Val Thr Cys Gln Ser Ser Glu Leu Ala Lys
 675 680 685
 Asp Ile Leu Val Pro Ser Phe Asp Pro Lys Asn Lys His Cys Val Phe
 690 695 700

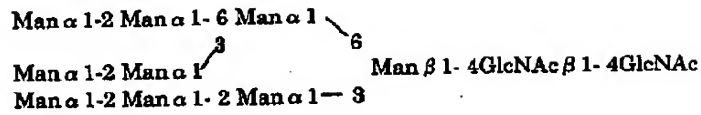
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 705 710 715 720
 Gln Arg Val Cys Pro Cys Arg Asp Phe Ile Lys Gly Gln Val Ala Leu
 725 730 735
 Cys Lys Asp Cys Leu
 740

Y } ° " E T " f A } X a " 難
 Y } P z a ¥ I " R ' m 難
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 Y } Q z a ¥ I " A fi n ^ 難
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 Y } R z G X | G ` 難
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 Y } S z f s | u " G } 難
 Ø B
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 ` q k N I ` h z æ 難
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 Y } T a z } T ` ~ " 難
 ` q k N I ` h z æ 難
 æ f • } - Ø B } T 難
 Y } U z { > { Æ - 難
 Ø B
 Y } V z { > { Æ - 難
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 } - 難
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 Ø B } X ` " A ` ç] • 難

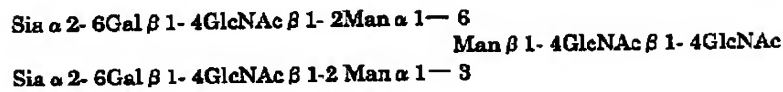
y) E

N-linked chain

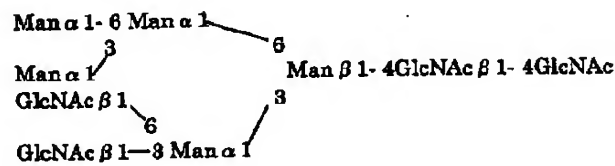
1) High mannose type



2) Complex type

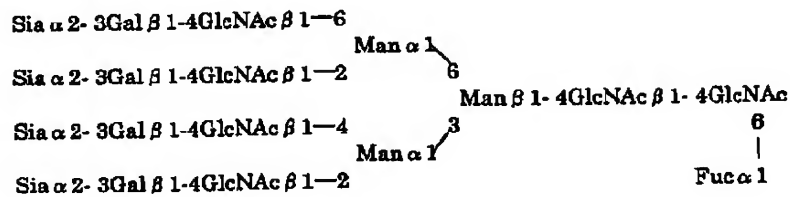


3) Hybrid type

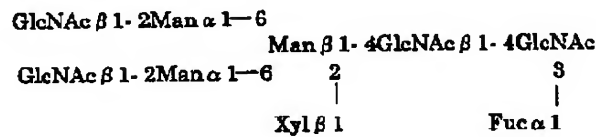


y) Q

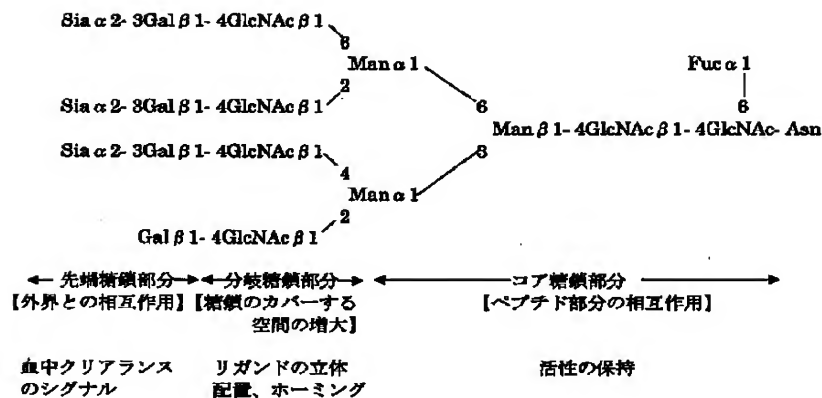
1) Mammalian cell type



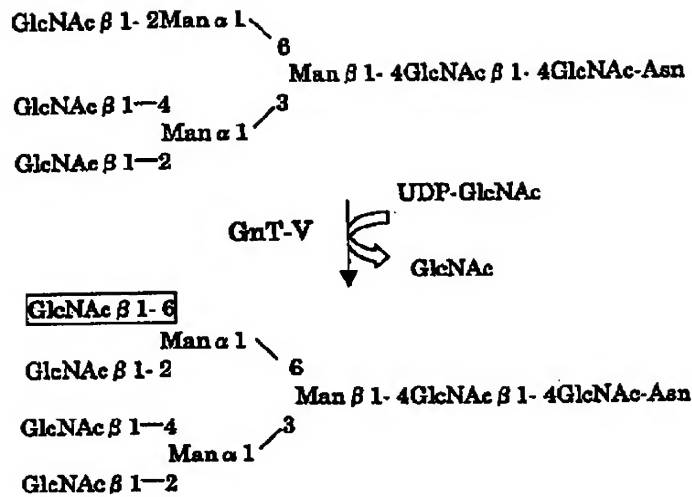
2) Plant cell Type



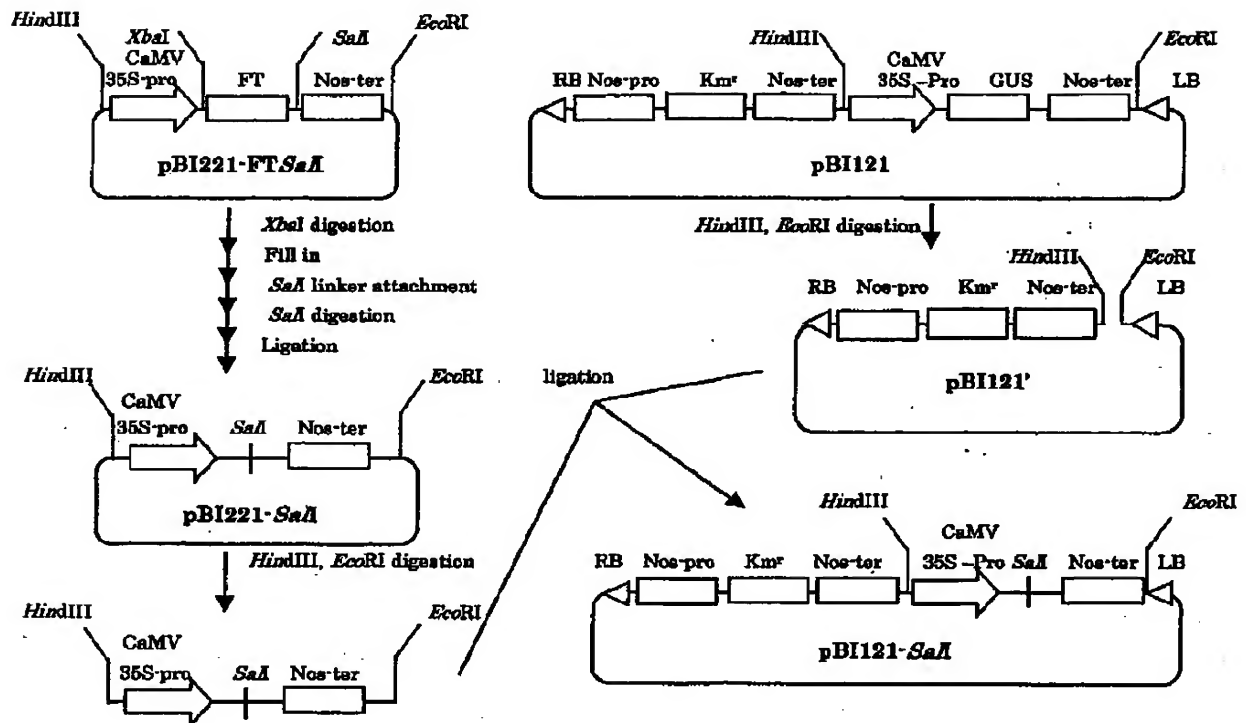
y) E



y } z



y } z



y } z

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y } x

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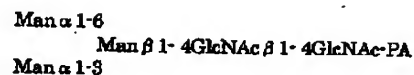
 CTATAG
 Leu***

$y \in \mathbb{N}$

PA057



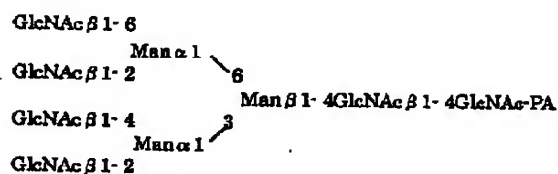
PA016



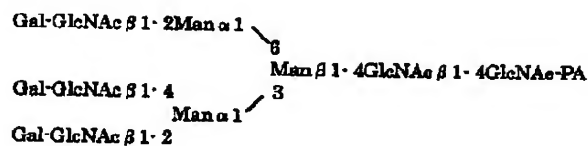
PA018



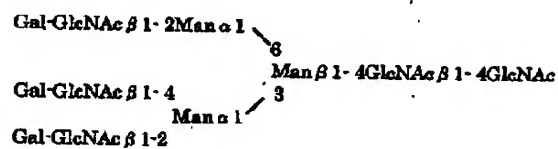
PA014



PA002

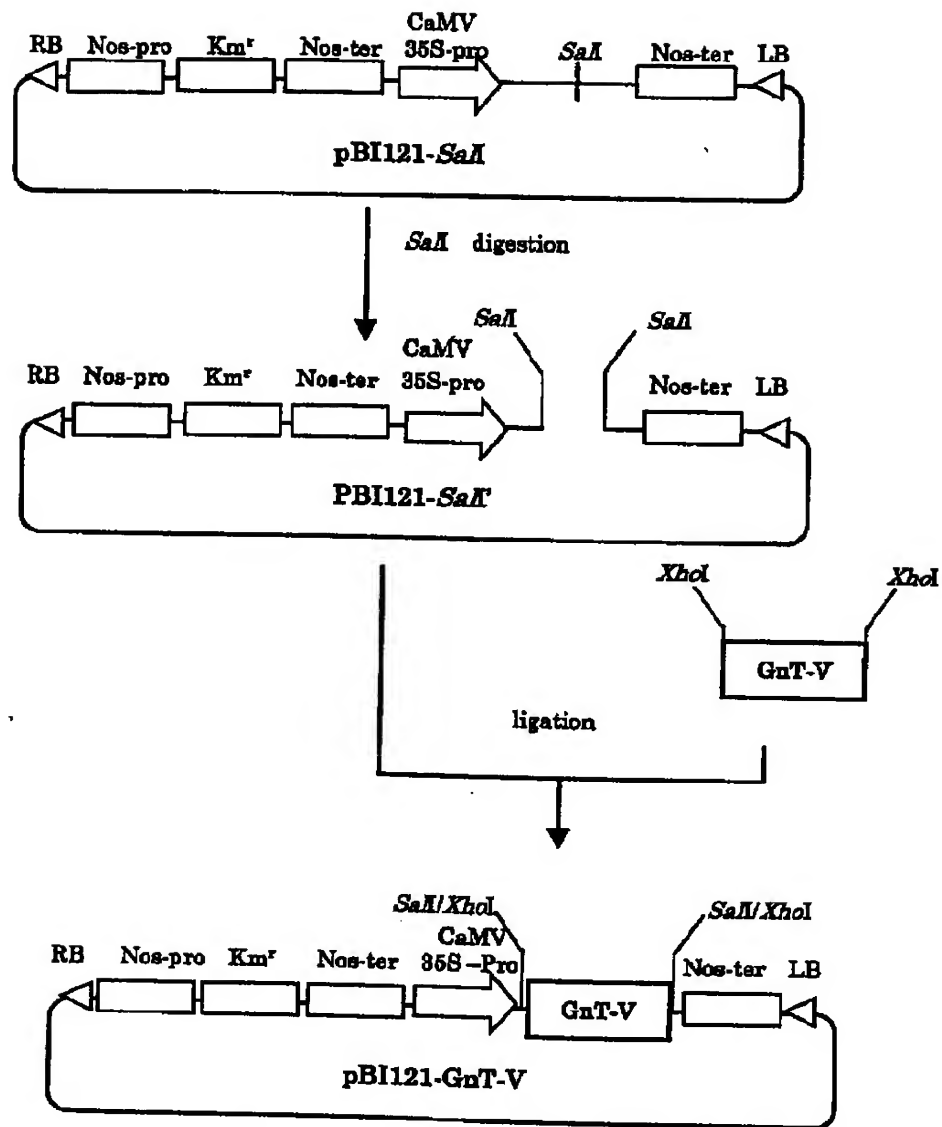


Sugar chain Gal3Gn3M8



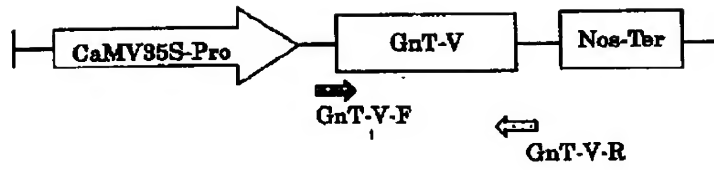
PA : Pridylamino(group)
Gal : Galactose
GlcNAc : N-Acetylglucosamine
Man : Mannose

y) W

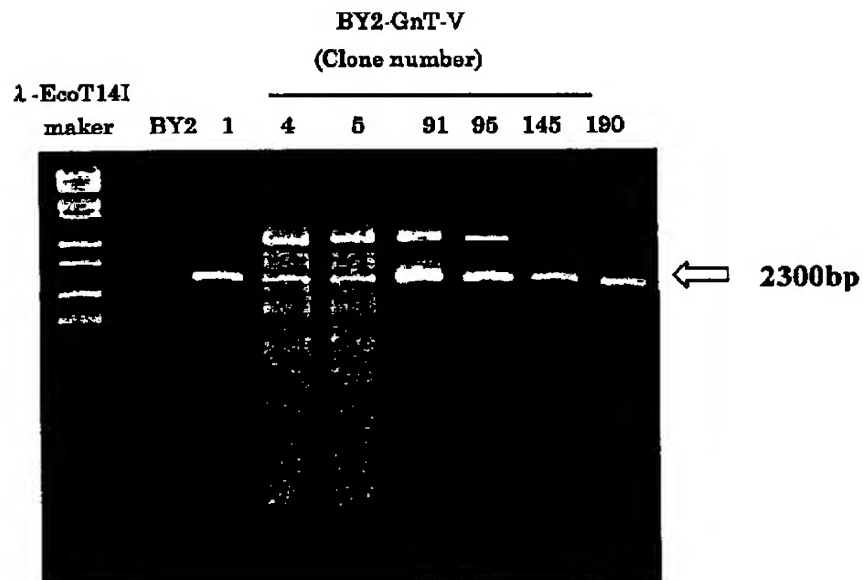


y) x

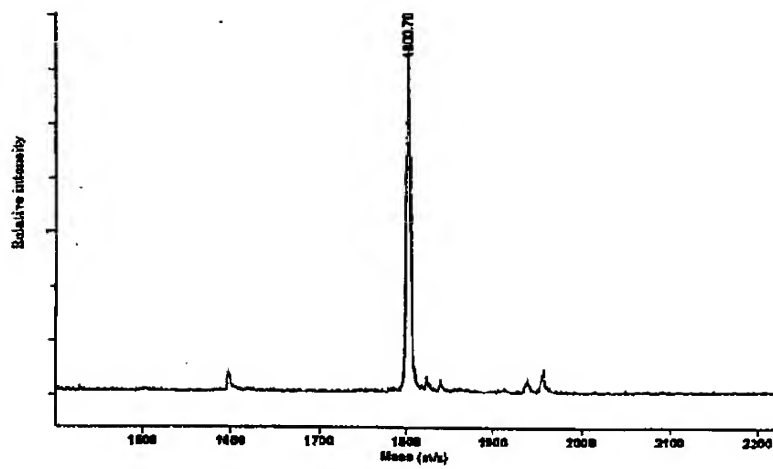
A



B

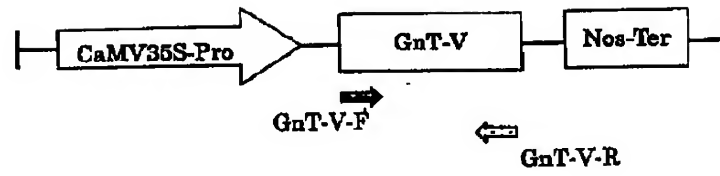


y) x

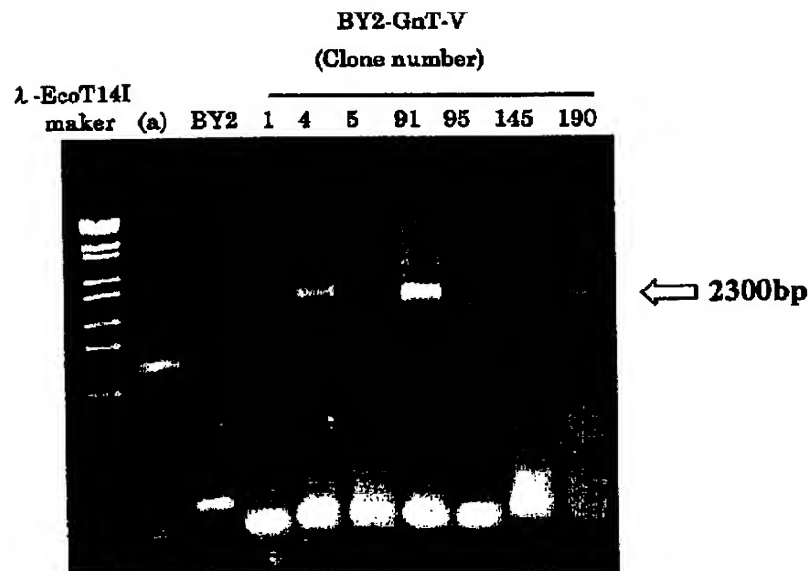


y } E

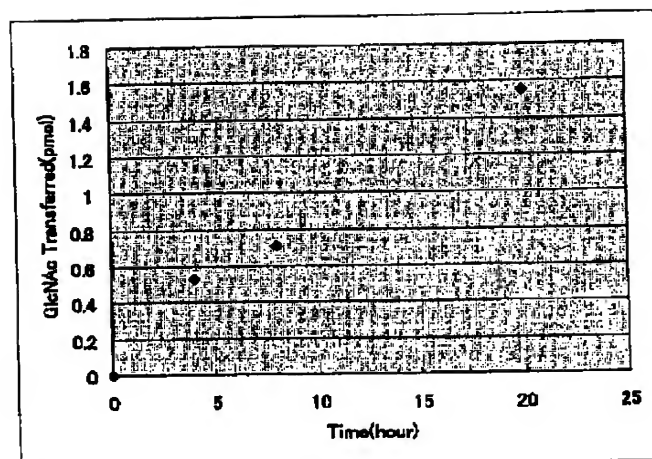
A



B



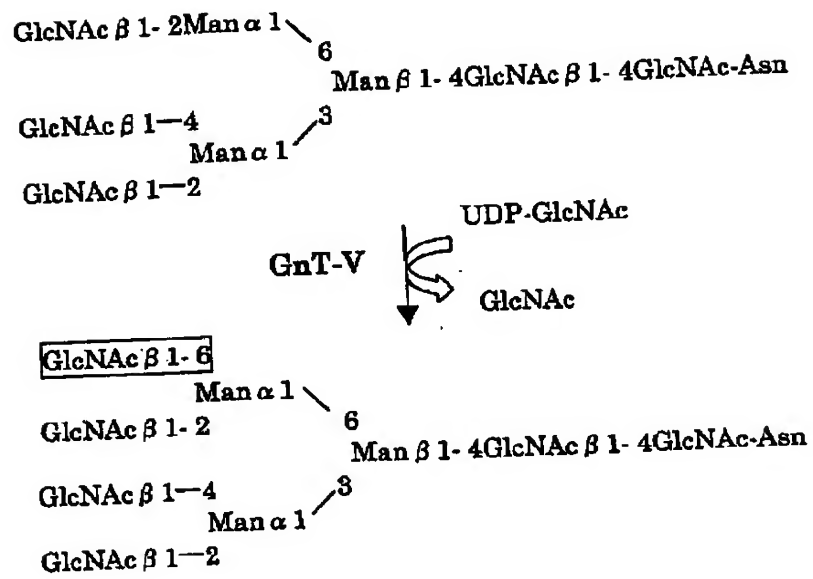
y } E



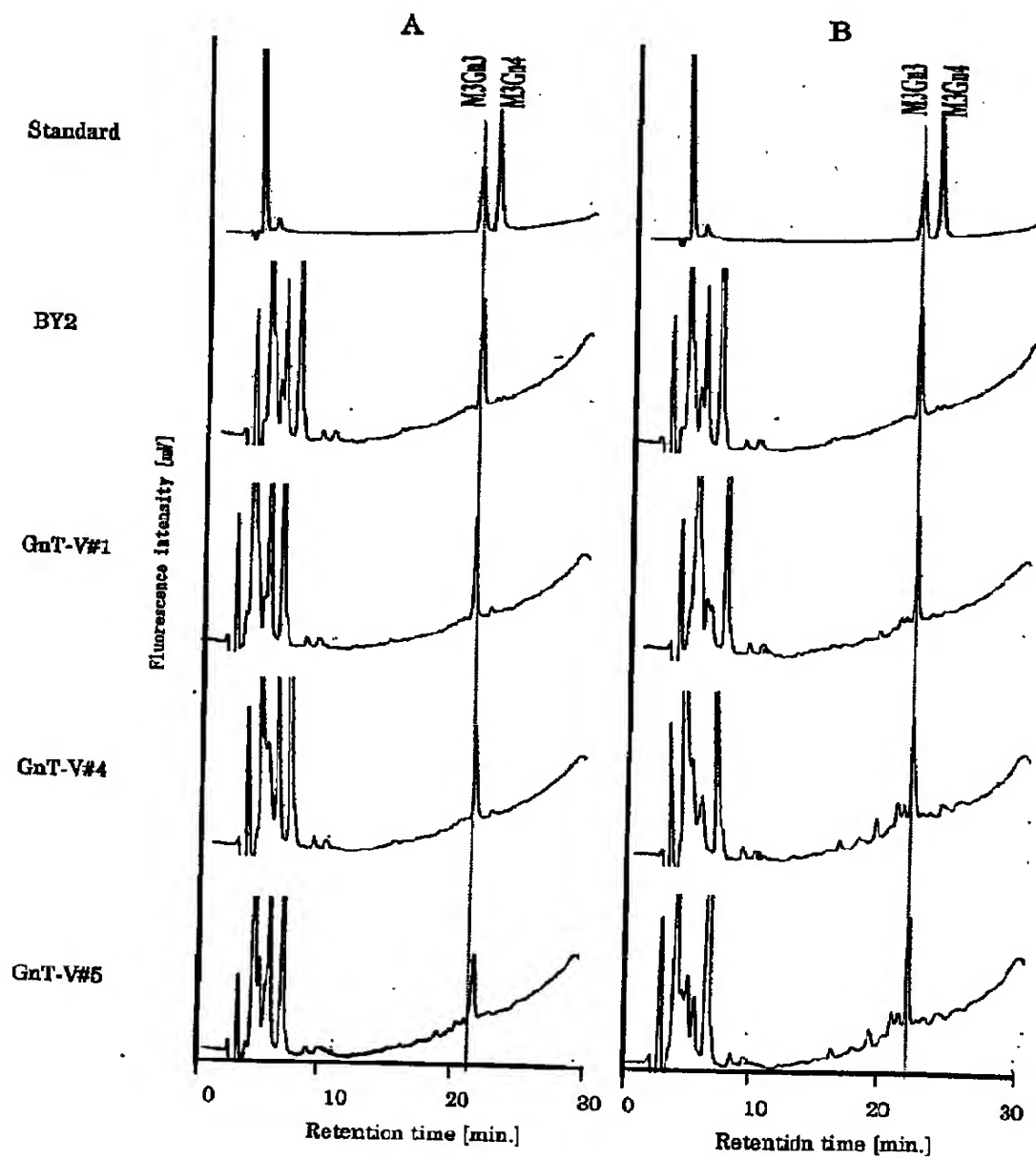
Y } E

(反応条件)

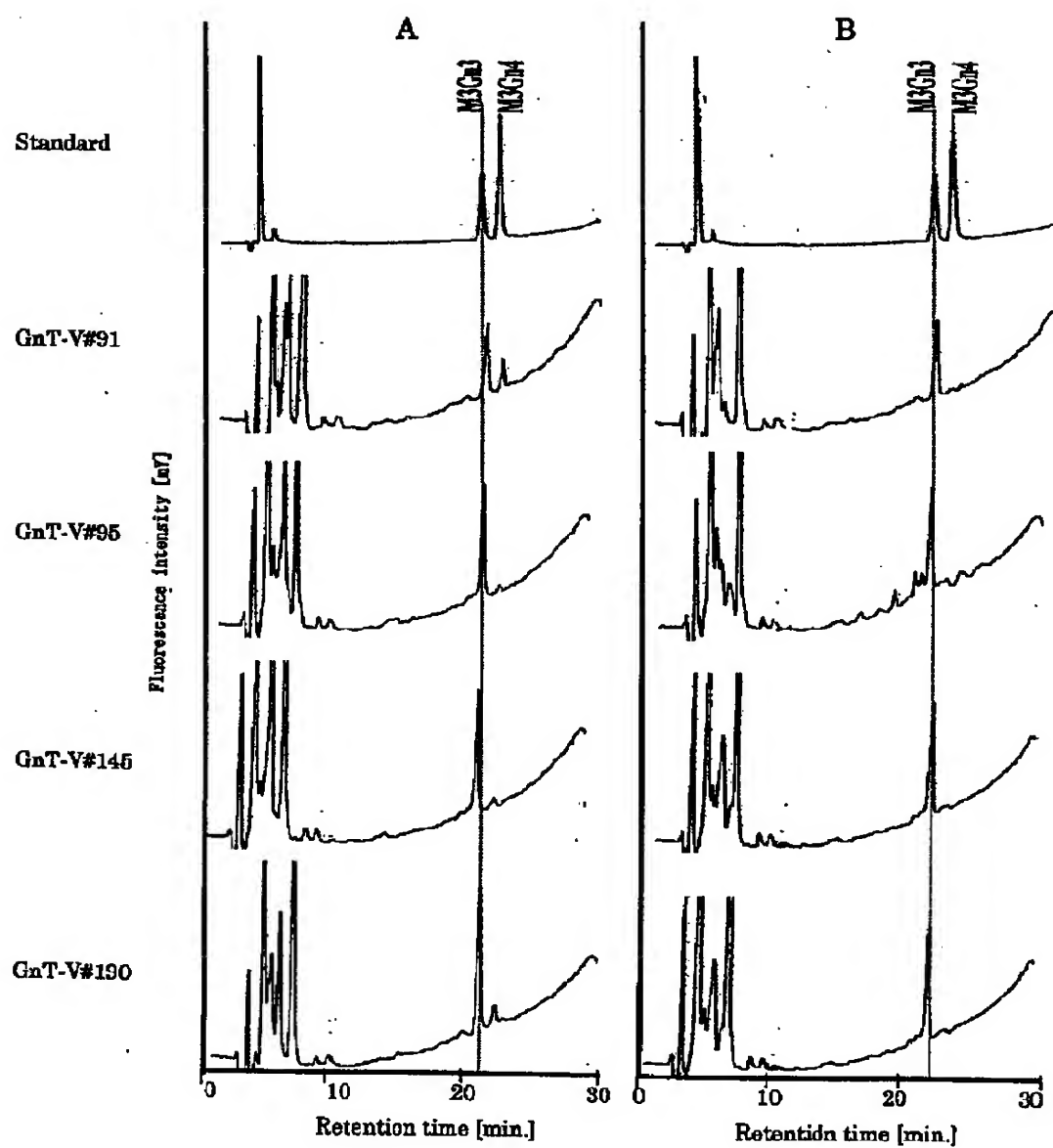
120mM	Mes バッファー (pH7.0)
300mM	N-アセチルグルコサミン
15mM	MnCl ₂
0.5%	TritonX-100
4pmol	PA 化糖鎖
308mM	UDP-N-アセチルグルコサミン
0.1~0.8 μg	タンパク質試料 (ミクロソーム画分)



y } P Ø

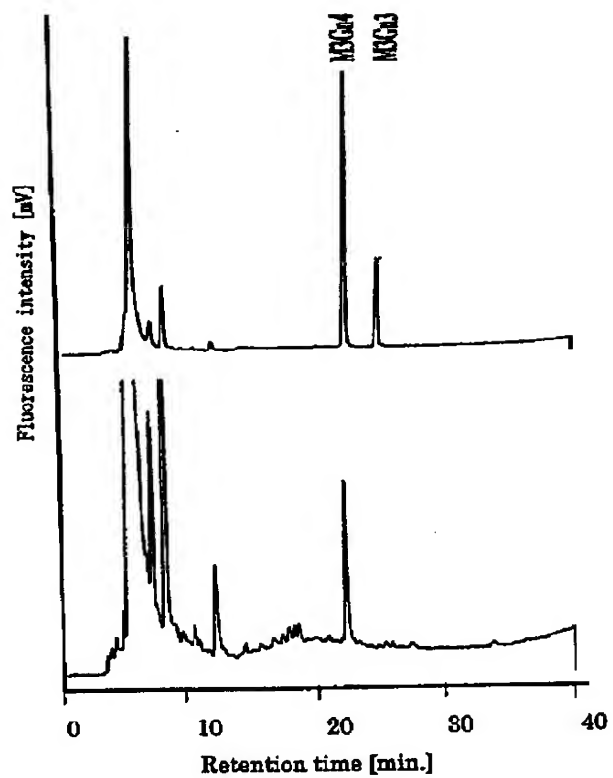


y } P @



Y } R

Standard

GrT-V#91
Product

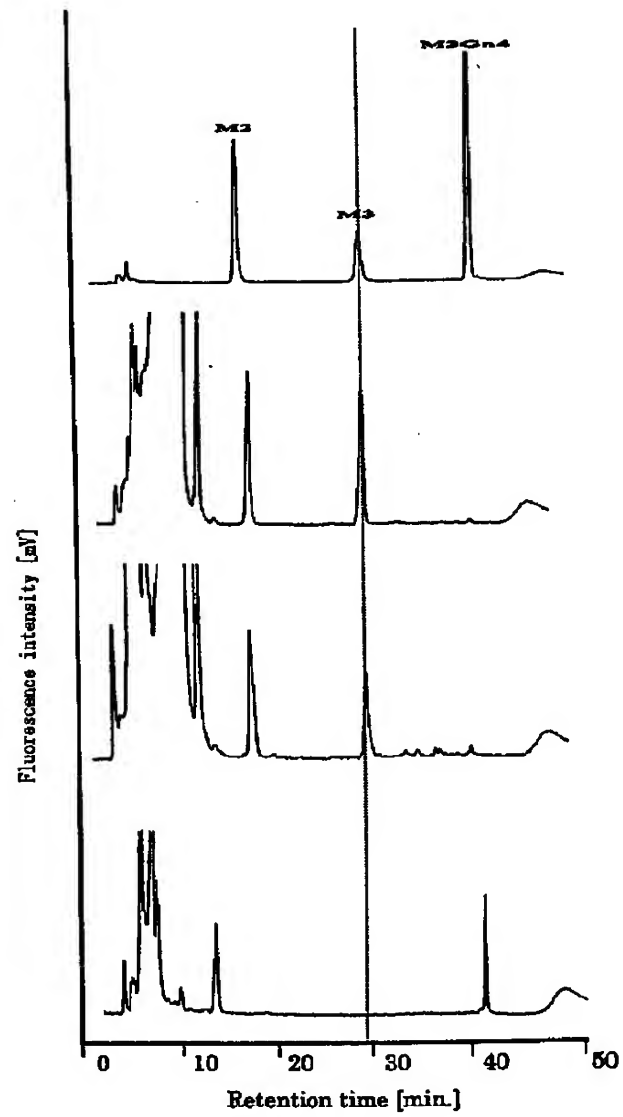
Y } E

Standard

PA014 N-ア
セチルヘキソ
サミニダーゼ
処理

BY2-GnT-V#91
Product N-アセチル
ヘキソサミニダーゼ
処理

BY2-GnT-V#91
Product 酵素未添
加



y } z

